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Diversity patterns of the rhizosphere and bulk soil microbial communities along an altitudinal gradient in an alpine ecosystem of the eastern Tibetan Plateau

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ABSTRACT

The diversity patterns and drivers of soil microbial communities in altitudinal gradients have recently received much attention. The rhizosphere is a focus of soil microbial communities, but the patterns and drivers of these communities have rarely been studied in alpine ecosystems. We used high-throughput Illumina sequencing to examine the community variations of bacteria, archaea and fungi between the rhizosphere and bulk soil along an altitudinal gradient in an *Abies fabri* (Mast.) community on Mount Gongga of the eastern Tibetan Plateau. Microbial alpha diversity and community structure varied significantly with altitude but not between the rhizosphere and bulk soil. Soil temperature and the carbon:nitrogen ratio were the primary drivers of the structures of the bacterial, archaeal and fungal communities, and altitude (geographic distance) contributed a small part ($< 3\%$) of the community variation, indicating that various edaphic factors were the key regulators of microbial-community variation. This consistency of the microbial communities between the rhizosphere and bulk soil in this alpine ecosystem could be attributed to low temperature and high nutrient content. The bacterial, archaeal and fungal communities were governed by specific environmental factors (total phosphorus content for bacteria; organic-carbon content, dissolved organic-carbon content, NH_4^+-N content and nutrient stoichiometry for archaea and NO_3^--N content for fungi). The distinct environmental responses of the microbial taxa suggested metabolic separation and resource preferences of the belowground communities, even within the small-scale spatial distances in this alpine ecosystem. Our study suggested that the ecosystem harbored many microbial taxa with diverse nutrient preferences and metabolic characteristics and could thus potentially tolerate the soil environmental variation under a scenario of climate change.

1. Introduction

Alpine ecosystems represent one of the most important components of the terrestrial system and provide many ecological services (Li et al., 2018). Climate, vegetation and soil properties in alpine ecosystems vary greatly over short spatial distances and altitudinal gradients (McCain, 2010). These changes will strongly affect the structures and functions of soil microbial communities (Shen et al., 2013; Lin et al., 2015). For example, geographic distance, soil pH, the carbon:nitrogen (C:N) ratio and vegetation type have been generally reported as the key drivers of the distributional patterns of soil microbes (Chen et al., 2017b; Li et al., 2018). Soil microorganisms play important roles in regulating biogeochemical cycles and maintaining ecosystem functions (Chen et al., 2017b). Also, soil microorganisms are more sensitive than plants and animals to environmental change (Shen et al., 2015). A better understanding of the patterns of geographic distribution and drivers of community assembly along environmental gradients of alpine ecosystems is therefore important for elucidating microbial processes, and for improving our predictions of the functions of alpine ecosystem in a changing climate.

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The rhizosphere, as a focus of microbial activity, plays an important role in microbial assembly because microbial-plant interactions and genetic exchanges are frequent there (Tkacz et al., 2015; Cui et al., 2018; Duan et al., 2018). Bacterial diversity is generally lower in the rhizosphere than the bulk soil (Marilley and Aragno, 1999), and microbial-community compositions differ greatly due to the strongly selective environment of rhizospheres (Kielak et al., 2010; Ai et al., 2012). Bulk soil has relatively oligotrophic conditions, with low rates of nutrient transformation and microbial activity, unlike the more active rhizosphere environment (Ai et al., 2012). Most studies of microbial communities in the alpine ecosystems, however, have focused on bulk soil (Shen et al., 2013; Chen et al., 2017b; Li et al., 2018). Microbial communities in alpine ecosystems have more stable habitats than communities in agricultural ecosystems with more annually variable conditions (Ai et al., 2012; Tkacz et al., 2015). These differences in physicochemical and biological properties suggest distinct differences in microbial communities between rhizosphere and bulk soil, and the responses of microbial communities to the rhizosphere conditions in alpine ecosystems may differ greatly from the responses in other ecosystems. The distributional patterns and drivers of microbial communities may consequently differ between rhizosphere and bulk soil in alpine ecosystems.

In addition to the influences of external conditions on microbial communities, the distinct responses of microbial taxa (bacteria, archaea and fungi) to environmental factors would lead to the variation of patterns of geographic distribution and differences in the drivers of community assembly (Zhang et al., 2017). Mounting evidences suggest that soil pH is a key regulator shaping the structures of bacterial and archaeal communities (J.T. Wang et al., 2015; Hu et al., 2016), but that plant diversity determines the structures of soil fungal communities over broad geographic scales (Chen et al., 2017a). Fungal diversity and richness decrease as altitude increases in alpine ecosystems such as the Tibetan Plateau (Margesin et al., 2009). These studies indicated that bacteria, archaea and fungi responded differently to environmental conditions. All these studies notably focused on a complete or large-scale altitudinal gradient, with relatively large elevational intervals and different vegetation types (Shen et al., 2013; Chen et al., 2017b; Li et al., 2018). The scale over which biodiversity is sampled will strongly influence the patterns observed (Green and Bohannan, 2006), so the effects of environmental factors on structuring microbial communities in a consistent ecosystem within a small-scale altitudinal gradient remain poorly known.

Metabolic characteristics differ greatly between bacteria and archaea, and both bacteria and archaea are very abundant and functionally important in terrestrial ecosystems. For example, ammonia-oxidizing archaea have unique mechanisms for nitrification, better adaptation to low-pH pressures, and strikingly lower ammonia requirements compared with ammonia-oxidizing bacteria (He et al., 2012; Hu et al., 2013). Fungal breakdown of plant materials rich in lignin and cellulose (i.e. lignocellulose) is centrally important to the cycling of terrestrial C due to the abundance of lignocellulose in above- and belowground systems (Meier et al., 2010). These differences in metabolic processes among bacteria, archaea and fungi and their important ecological functions (Nemergut et al., 2010; Meier et al., 2010), indicate that further understanding of the responses of bacterial, archaeal and fungal taxa to environmental conditions in small-scale altitudinal gradients with the same vegetation type is necessary for accurately assessing the altitudinal patterns of distribution and drivers of community assembly in alpine ecosystems and can improve the resolution and precision of our knowledge.

Mount Gongga is the highest mountain on the eastern boundary of the Tibetan Plateau. It has steep slopes, distinct vegetation and relatively low environmental temperature (He and Tang, 2008). We previously reported that the soil of this area has abundant organic matter and sources of available N and phosphorus (P), with a C:N:P ratio of 556:22:1 for the O horizon (Bing et al., 2016), which provide abundant energy and nutrients for the local microorganisms and plants. These conditions provide a natural platform for identifying geographic distributional patterns and drivers of community assembly along an altitudinal gradient. These conditions are also helpful for assessing potential microbial responses to climate change, with a strategy of space-for-time substitution. We investigated the altitudinal distributional patterns and driving factors of the bacterial, archaeal and fungal communities in the rhizosphere and bulk soil along an altitudinal gradient from 2800 to 3500 m a.s.l. containing the same vegetation type (*Abies fabri* Mast.) on Mount Gongga. We hypothesized that: (1) the pattern of microbial-community diversity would not vary significantly along the small-scale altitudinal gradient in an *A. fabri* community due to the small spatial scale and consistent vegetation, (2) the microbial communities would not distinctly vary between the rhizosphere and bulk soil because of the low environmental temperature and sufficient resources in the alpine ecosystems, and (3) the driving factors of the bacterial, archaeal and fungal taxa would differ due to the differences in their metabolic processes, even within a short distance.

2. Materials and methods

2.1. Study area and soil sampling

The study area was in the Hailuogou catchment of Mount Gongga (29°30′–30°20′N, 101°30′–102°15′E; 2800–3500 m a.s.l.) (Fig. 1). The mountain is in the transition zone of the Tibetan Plateau frigid zone and the warm-humid subtropical monsoon zone and is the highest mountain in the Hengduan Mountains. The climate in the area is mainly controlled by the Asian monsoon. Mean annual temperature and precipitation are 4.2 °C and 1947 mm, respectively (Wu et al., 2013). The soil has mainly developed from glacial debris and colluvial deposits derived from weathered Cenozoic feldspar granite and Permian quartz schist. The specific types of soil and vegetation with altitude have been described elsewhere (Bing et al., 2016).

Soil was collected from four altitudes (2800, 3000, 3200, and 3500 m) in a subalpine dark coniferous forest dominated by *A. fabri*. Three 10 × 10 m plots were established at each altitude in October 2017. The plots were separated by > 15 m and were considered as true replicates (Mariotte et al., 1997). Bulk and rhizosphere soil were collected from each plot. The bulk soil, not directly attached to the root systems of *A. fabri*, was collected by removing several roots and gently shaking them to release the soil. The rhizosphere soil, tightly adhered to the root surface, was then physically brushed from the root surfaces with a sterile soft-bristled paintbrush. Each soil sample was divided into two subsamples. One subsample was immediately placed in an ice box, transported to the laboratory, and then stored at −80 °C for the extraction of genomic DNA. The other subsample was passed through a 2-mm sieve and air-dried for physicochemical analysis.

2.2. Soil physiochemical analysis

The amount of soil moisture was determined by oven-drying 10 g of fresh soil at 105 °C for 48 h. Soil pH was measured for a 1:2.5 soil:water (w/v) mixture using a meter with a glass electrode (InsMark™ IS126, Shanghai, China). Soil organic-C (SOC) content was analyzed using the dichromate oxidation method; approximately 0.10 g of air-dried soil was digested with 5 ml of 0.8 M $K_2Cr_2O_7$ and 5 ml of H_2SO_4 for 5 min at 170–180 °C and was then titrated using 0.2 M $FeSO_4$. Dissolved organic C was extracted with 0.5 M K_2SO_4 and shaken for 60 min at 200 rpm on a reciprocal shaker, and the extracts were measured using a Liqui TOCII analyzer (Elementar, Germany) (Jones and Willett, 2006). Total N (TN) content was measured by the Kjeldahl method (Bremner and Mulvaney, 1982). In detail, approximately 0.700 g of air-dried soil was digested with 1.85 g of a mixed catalyst (100:10:1 K_2SO_4:$CuSO_4$:Se) and 5 ml of H_2SO_4 for 45 min at 385 °C and was then titrated using 0.02 M HCl. Soil NO_3^--N and NH_4^+-N contents were measured using a Seal Auto

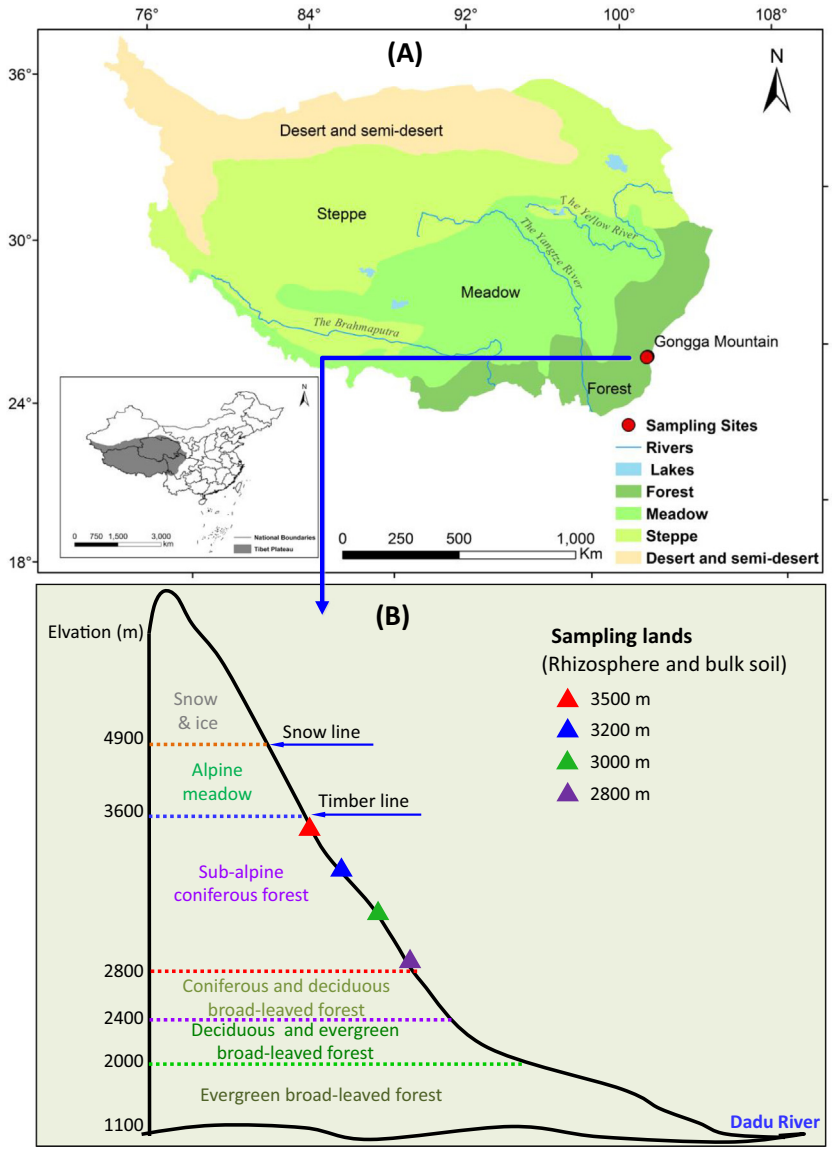

Fig. 1. Maps of the Tibetan Plateau and study area (A), and a graph of the sampling sites along the altitudinal gradient on Mount Gongga (B).

Analyzer after extraction with 2 M KCl with a 1:5 ratio. Total P (TP) and available P (AP) were extracted with H_2SO_4-$HClO_4$ and sodium bicarbonate (Olsen and Sommers, 1982), respectively, and then determined by the molybdenum blue method using an ultraviolet spectrophotometer (Hitachi UV2300).

2.3. DNA extraction, amplification and MiSeq sequencing

DNA was extracted from 0.25 g of soil using the FastDNA SPIN Kit for Soil (Q-BIOgene, Carlsbad, USA) following the manufacturer's instructions. The quality and quantity of the extracted DNA was assessed using an automatic microplate reader (BioTek ELX 800, USA). The integrity of the DNA extracts was confirmed by 1% agarose gel electrophoresis. The primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Huse et al., 2008) were used to amplify the V3–V4 hypervariable region of the bacterial 16S rRNA gene. The primers Arch349F (5′-GYGCASCAGKCGMGAAW-3′) and Arch806R (5′-GGACTACVSGGGTATCTAAT-3′) (Takai and Horikoshi, 2000) were used to amplify the V3–V4 hypervariable regions of the archaeal 16S rRNA gene. The primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) (Gardes and Bruns, 1993) were used to amplify the fungal ITS1 region. The PCR reactions were performed in a thermal cycler (ABI GeneAmp 9700) at a volume of 20 μl and undergone 5 cycling procedure. Successful PCR amplification was verified by 2% agarose gel electrophoresis. The three PCR products were pooled, purified by gel extraction and quantified using the AxyPrepDNA gel extraction kit (AXYGEN Corporation, USA) and the QuantiFluor™-ST blue fluorescence quantitative system (Promega Corporation, USA). The purified PCR products were then mixed at equimolar ratios for sequencing on an Illumina HiSeq PE150 system (Illumina Corporation, USA) by Biomarker Technologies Co, LTD.

2.4. Bioinformatics analysis

Primer sequences were trimmed after the raw sequences were denoised, sorted and separated using Trimmomatic (version 0.33). The remaining sequences were filtered for redundancy, and all unique sequences for each sample were then clustered into operational taxonomic units (OTUs) at similarities of 97%. Low-abundance OTUs were eliminated from the OTU table if they did not present a total of at least two counts across all samples in the experiment. The taxonomic identify (species level) of representative sequences for each OTU was determined using the Silva reference database (http://www.arb-silva.de)

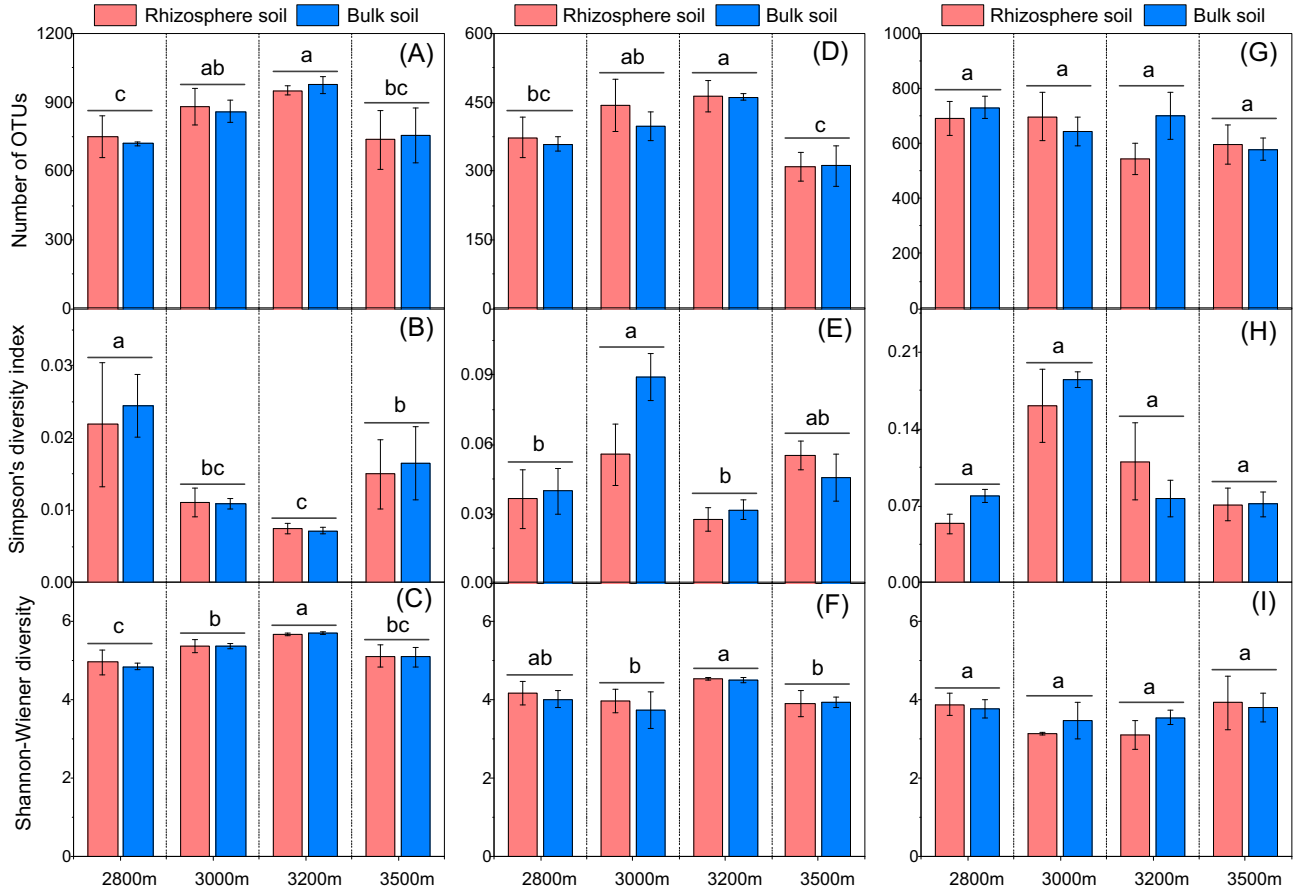

Fig. 2. Differences in bacterial (A, B and C), archaeal (D, E and F) and fungal (G, H and I) alpha diversity among the altitudes. All data are presented as the mean ± standard error. Lowercase letters (a, b and c) indicate that means are significantly different ($P < 0.05$) among different altitudes within rhizosphere and bulk soil; whereas there are no significant differences in microbial alpha diversity between the rhizosphere and bulk soil ($P > 0.05$).

for the 16S rRNA genes and the Unite reference database (http://unite.ut.ee/index.php) for the ITS using the RDP naïve Bayesian classifier with the BLAST tool in QIIME (http://qiime.org/index.html). Alpha diversity was calculated using the Shannon-Wiener and Simpson's diversity indices by the 'diversity' function in the R (v.3.3.2) Vegan package (version 2.4-4; Oksanen et al., 2013). The relative abundances of the microbes were determined as percentages.

2.5. Statistical analysis

Two-way ANOVAs were used to analyze the effects of altitude, location (bulk soil and rhizosphere) and their interaction on the soil properties and microbial alpha diversities (number of OTUs and Simpson's diversity and Shannon-Wiener indices). A Pearson correlation analysis assessed the association between microbial alpha diversity and environmental factors. A value of $P < 0.05$ was considered significant. The heterogeneity of the variance was tested, and the original data were normalized by log-transformation or standardization prior to analysis when necessary. These analyses were performed using R (v.3.3.2).

The structures of the bacterial, archaeal and fungal communities were visualized by principal coordinates analyses (PCoA) based on Bray-Curtis dissimilarity matrices using the Vegan package (Oksanen et al., 2013). The difference of microbial-community structure between two altitudes was identified by analysis of similarities (ANOSIM). The effects of altitude, location and their interaction on the microbial Bray-Curtis dissimilarity were tested by a two-way permutational multivariate analysis of variance using the Adonis function in the Vegan package (https://cran.r-project.org/web/packages/vegan/index.html). The most significant factors shaping the structures of the microbial communities were determined by a canonical correspondence analysis (CCA) and a Monte Carlo permutation test via the Hellinger transferred data of microbial species and the data of environmental factors standardized using the Vegan package. A Mantel test was used to assess the correlations of microbial communities and environmental variables and geographic distance using the Vegan package. A partial Mantel test in the Vegan package was used to control the covarying effects of various factors. Significant environmental variables identified by the partial Mantel test were selected to construct an environmental matrix for conducting a variation-partitioning analysis to determine the relative importance of the environmental variables and geographic distance in explaining the microbial-community compositions identified by a redundancy analysis using the varpart function in the Vegan package.

3. Results

3.1. Soil characteristics along the altitudinal gradient

Most soil parameters differed significantly between the rhizosphere and bulk soil along the altitudinal gradient (Tables S2 and S3). The two-way ANOVAs indicated that the SOC, DOC and NH_4^+-N contents, the C:P and N:P ratios and soil temperature were significantly higher at 2800 and 3000 m than at 3200 and 3500 m ($P < 0.05$). The NO_3^--N, NH_4^+-N and TP contents and the C:N and C:P ratios were strongly affected by both altitude and location and their interaction ($P < 0.05$). The Pearson correlation analysis identified strong correlations among soil properties, nutrient stoichiometry and soil temperature (Table S4).

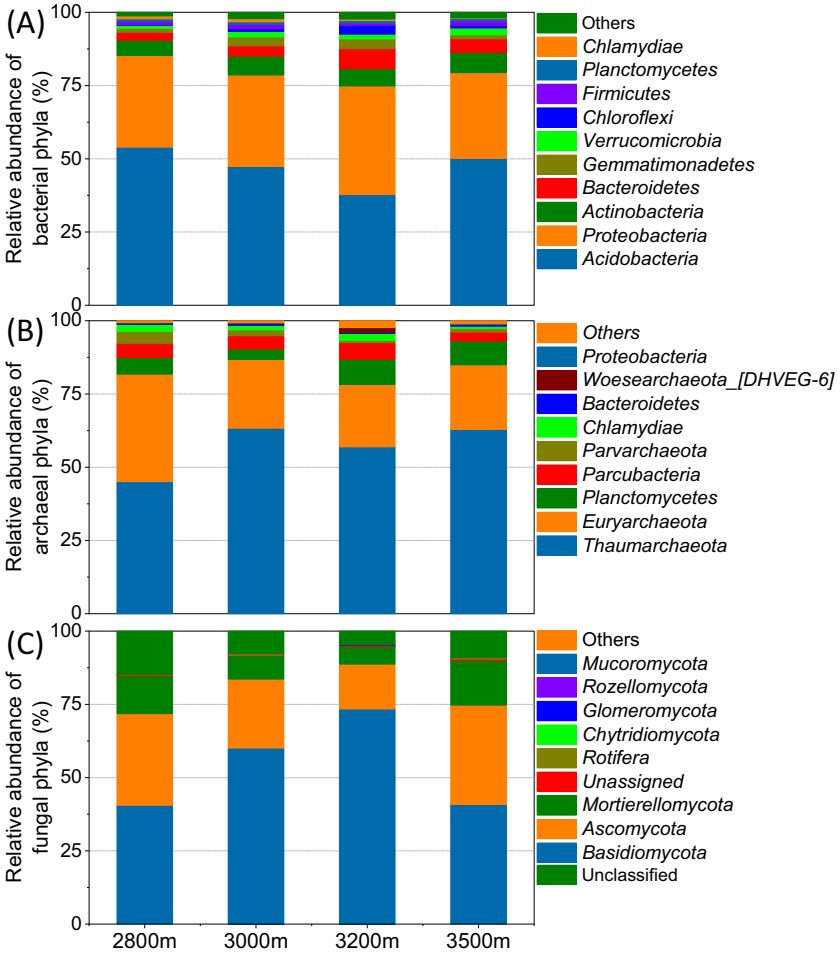

Fig. 3. Relative abundance of the dominant bacterial, archaeal and fungal taxa among the altitudes. (A) Relative abundance of bacterial phyla (%), (B) relative abundance of archaeal phyla (%), (C) relative abundance of fungal phyla (%).

3.2. Microbial alpha diversity and community composition

A total of 4,065,875 high-quality microbial sequences were identified from all soil samples: 1,482,852 bacterial, 1,112,142 archaeal and 1,470,881 fungal sequences (Table S1). The bacterial, archaeal and fungal sequences were clustered into 1271, 753 and 2043 OTUs, respectively. For the bacteria and archaea communities, the numbers of OTUs and the Shannon-Wiener and Simpson's diversity indices indicated significant differences along the altitudinal gradient, but these indices did not differ significantly between rhizosphere and bulk soil (Fig. 2 and Table S5). In contrast, the fungal alpha diversities were similar among the sampling sites.

The dominant bacterial phyla at 2800, 3000, 3200 and 3500 m were *Acidobacteria* (54.0, 47.5, 37.8 and 50.1%, respectively) and *Proteobacteria* (31.3, 31.1, 37.1 and 29.3%, respectively) (Fig. 3A). The most abundant archaeal phyla at 2800, 3000, 3200 and 3500 m were *Thaumarchaeota* (45.0, 63.3, 56.9 and 63.0%, respectively) and *Euryarchaeota* (36.7, 23.5, 21.4 and 22.0%, respectively) (Fig. 3B). The most abundant fungal phyla at 2800, 3000, 3200 and 3500 m were *Basidiomycota* (40.5, 60.1, 73.4 and 40.8%, respectively) and *Ascomycota* (31.2, 23.5, 15.3 and 33.9%, respectively) (Fig. 3C). Other phyla such as *Chytridiomycota* and *Glomeromycota* accounted for only a minor fraction of the fungal-community composition.

3.3. Effect of environmental variables on microbial alpha diversity and microbial-community structure

Altitude rather than location (rhizosphere or bulk soil) affected the bacterial and archaeal alpha diversities (Table S5). The Pearson correlation analysis found that all environmental factors except soil temperature were significantly correlated with bacterial alpha diversities (Table S6). The archaeal alpha diversities were significantly correlated only with NO_3^--N and AP contents, the C:N ratio and pH. The number of fungal OTUs was significantly correlated with SOC, DOC, TN and NH_4^+-N contents, N:P ratio, soil moisture and temperature.

The PCoA and ANOSIM found that the structures of the bacterial, archaeal and fungal communities differed significantly among the four altitudes (Table 2 and Fig. 4). The Adonis analysis also indicated that altitude significantly affected microbial-community structure ($P < 0.001$; Table 1). Both the CCA and Monte Carlo permutation test indicated that altitude, the C:N ratio and soil temperature concurrently affected the structures of the bacterial, archaeal and fungal communities. Specifically, the correlations indicated that the TP content significantly affected bacterial-community structures. SOC, DOC and NH_4^+-N contents, and the C:P and N:P ratios regulated archaeal-community structures. NO_3^--N content had a strong impact on fungal-community structures ($P < 0.05$; Table 3 and Fig. 5).

3.4. Associations of microbial beta diversity with environmental variables and geographic distance

The partial Mantel test found that environmental factors (e.g., SOC and DOC) and geographic distance (i.e., the relative distance between the sampling sites) were both significantly correlated with the dissimilarity of the bacterial, archaeal and fungal communities (all $P < 0.05$; Table 4), indicating a significant distance-decay relationship. The

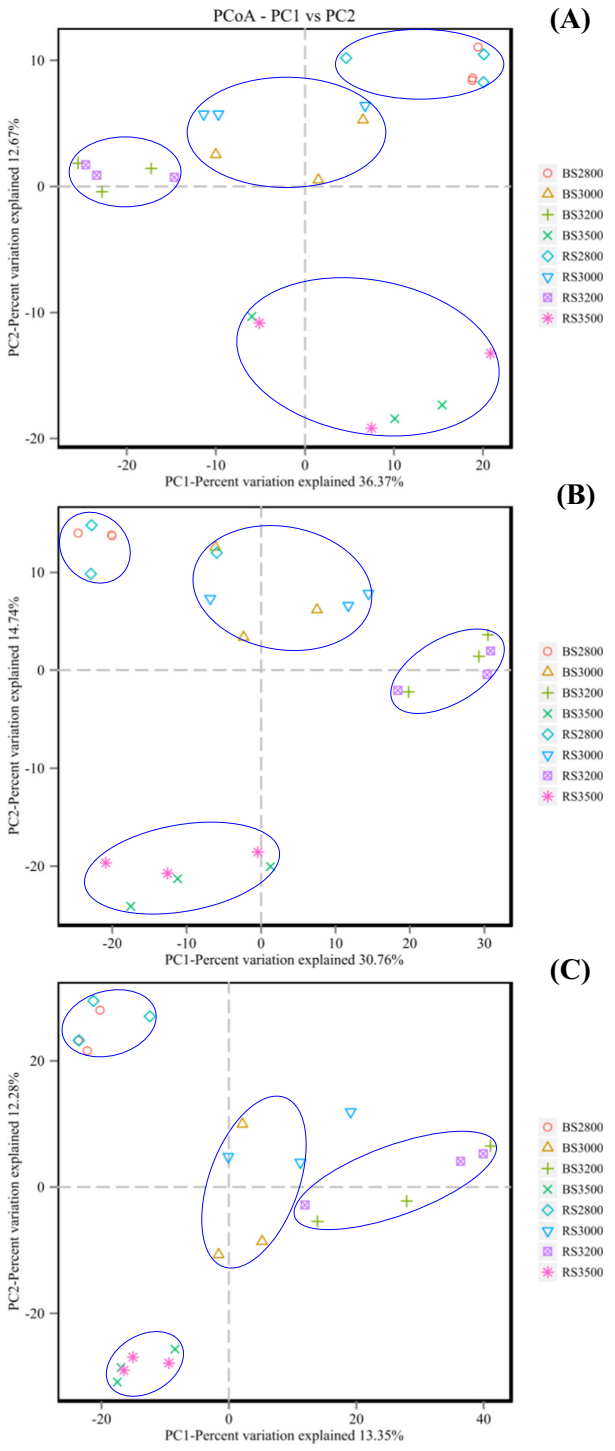

Fig. 4. Principal coordinates analysis (PCoA) of bacteria (A), archaeal (B) and fungal (C) community structures in the rhizosphere and bulk soil among the altitudes.

variation-partitioning analysis found that the environmental factors were the main contributors to the dissimilarities of the bacterial, archaeal and fungal communities, explaining 53.9, 47.9 and 24.9% of the variation, respectively (Fig. 6). Geographic distance explained only a small percentage of the microbial-community dissimilarity. For example, geographic distance explained only 0.6% of the fungal-community dissimilarity. By comparison, the environmental factors were important predictors of fungal beta diversity (Fig. 6). These results indicated that the contemporary environment played a more important role than geographic distance in shaping the composition of the microbial communities.

Table 1
Two-way permutational multivariate analysis of variance (PERMANOVA) (Adonis analysis) showing the effects of altitude, location (rhizosphere and bulk), and their interaction on microbial-community structure.

Factors	df	*F*	*P*
Bacteria			
Altitude	3	7.94	**0.001*****
Location	1	1.25	0.260
Altitude ∗ location	3	0.46	0.975
Residuals	16		
Total	23		
Archaea			
Altitude	3	6.91	**0.001*****
Location	1	1.36	0.205
Altitude ∗ location	3	0.61	0.923
Residuals	16		
Total	23		
Fungi			
Altitude	3	3.29	**0.001*****
Location	1	0.41	0.997
Altitude ∗ location	3	0.35	1.000
Residuals	16		
Total	23		

Note: df, degrees of freedom.
*** $P < 0.001$.

4. Discussion

4.1. Differences in microbial alpha diversity and community structure along the altitudinal gradient

Our results indicated that the alpha diversities and community structures of the soil microbes on Mount Gongga varied markedly along an altitudinal gradient containing the same type of vegetation, which did not support our first hypothesis. The soil properties and spatial attributes associated with altitude greatly affected the composition of the belowground communities. Other studies have also reported variations of microbial communities along altitudinal gradients (Yang et al., 2014; Guo et al., 2015; J.T. Wang et al., 2015). These differences in the microbial communities can be ascribed to differences in the environmental conditions along the gradients. A pattern of decreased microbial diversity with altitude emphasizes the importance of environmental variables (J.T. Wang et al., 2015; Bryant et al., 2008). Furthermore, environmental fluctuations are larger in areas at low altitude, but areas at high altitude feature many restraining factors such as low temperature, nutrient and water availability and thermal energy and high viscosity (Jansson and Taş, 2014).

Only microbes (psychrotolerant or psychrophilic) with structural and functional adaptations to the harsh environmental conditions can survive at high altitudes. For example, microbes can evolve multiple strategies such as dormancy or the production of specialized proteins to survive at low temperatures (Jansson and Taş, 2014). Our correlation analysis between the matrix of environmental variables and community structures indicated that microbial-community structure was closely correlated with the environmental factors, suggesting that the Bass-Becking hypothesis may apply. The Bass-Becking hypothesis states that "everything is everywhere, but, the environment selects" and assumes that microbes are ubiquitous and that environmental factors contribute decisively to structuring microbial-community composition (O'Malley, 2007). The environmental factors in our study had obvious distributions at the various altitudes (Tables S2 and S3) and greatly influenced microbial-community composition (Tables 3 and 4, Figs. 5 and 6). The apparent diversity patterns of the various microbial taxa highlighted the importance of these soil environmental factors in regulating

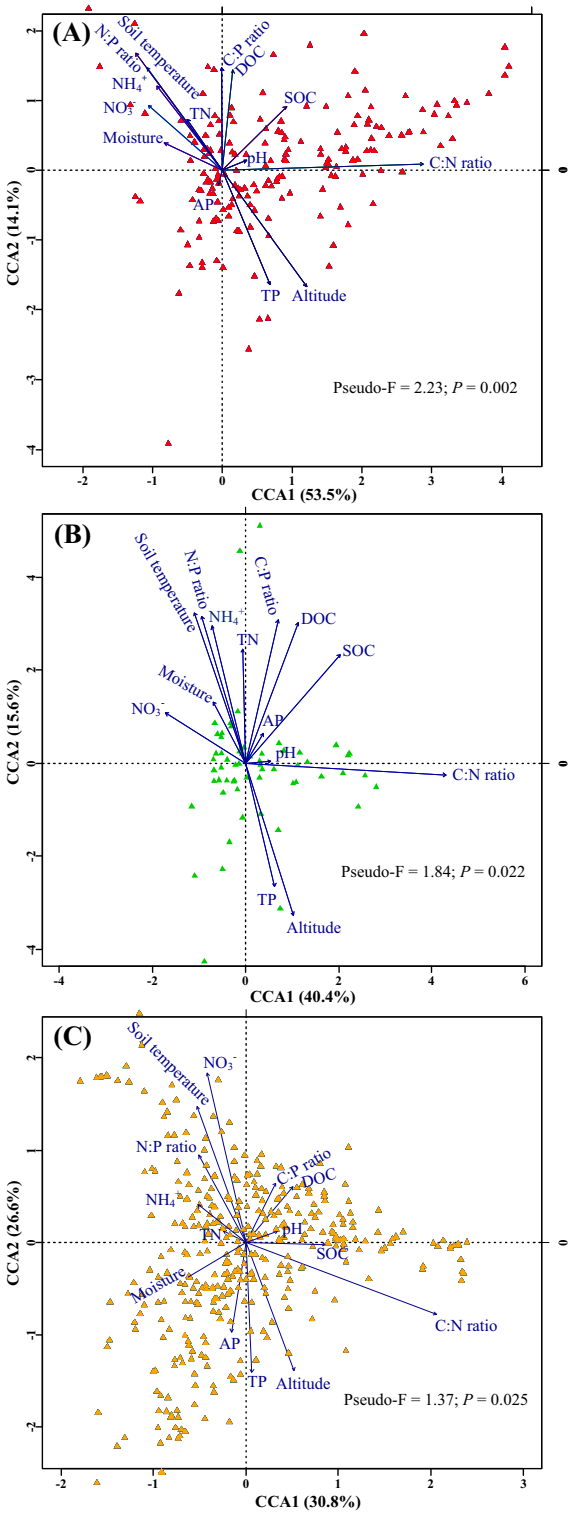

Fig. 5. Canonical correspondence analysis (CCA) used to identify the relationships among the bacterial (A), archaeal (B), and fungal (C) populations (trilateral), environmental factors and geographic distance. SOC, soil organic-carbon content; DOC, dissolved organic-carbon content; TN, total nitrogen content; TP, total phosphorus content; AP, available P content; C:N ratio, the ratio of SOC to TN; C:P ratio, the ratio of SOC to TP; N:P ratio, the ratio of TN to TP; Moisture, soil moisture content.

microbial distribution. Fungal alpha diversity particularly did not differ significantly among the altitudes, possibly due to an unexpectedly efficient dispersal by known agents, including wind and birds (Egan et al., 2014). Also, many fungal species can form spores (Harrison, 2005), so a bank of propagules might be formed to efficiently exploit even harsh environmental conditions (Davison et al., 2015).

The microbial alpha diversity and community structures did not differ significantly between the rhizosphere and bulk soil, supporting our second hypothesis. Other studies of grassland and agricultural soil,

Table 2
Analysis of similarities (ANOSIM) showing the differences of the microbial-community structure between the altitudes.

Altitude	Bacteria		Archaea		Fungi	
	R^2	P	R^2	P	R^2	P
2800–3000 m	0.669	0.007**	0.691	0.006**	0.713	0.003**
2800–3200 m	0.985	0.002**	0.967	0.003**	0.963	0.001***
2800–3500 m	0.693	0.003**	0.776	0.005**	0.994	0.001***
3000–3200 m	0.735	0.002**	0.659	0.003**	0.378	0.005**
3000–3500 m	0.639	0.03*	0.591	0.003**	0.507	0.005**
3200–3500 m	0.9	0.001***	0.917	0.002**	0.859	0.003**

*** $P < 0.001$.
** $P < 0.01$.
* $P < 0.05$.

Table 3
Relationships of bacterial, archaeal and fungal community compositions with environmental variables identified by Monte Carlo permutation tests.

Factors	Bacteria		Archaea		Fungi	
	R^2	P	R^2	P	R^2	P
Altitude	0.288	**0.038***	0.361	**0.007****	0.294	**0.026***
SOC	0.053	0.573	0.253	**0.049***	0.056	0.543
DOC	0.079	0.430	0.312	**0.017***	0.049	0.588
TN	0.015	0.846	0.190	0.108	0.006	0.943
NO_3^--N	0.221	0.076	0.097	0.369	0.299	**0.024***
NH_4^+-N	0.080	0.429	0.286	**0.039***	0.036	0.667
TP	0.270	**0.039***	0.231	0.082	0.165	0.133
AP	0.064	0.520	0.016	0.856	0.079	0.438
C:N ratio	0.384	**0.009****	0.374	**0.011***	0.384	**0.008****
C:P ratio	0.090	0.376	0.309	**0.024***	0.042	0.630
N:P ratio	0.151	0.168	0.335	**0.019***	0.097	0.357
Moisture	0.035	0.679	0.065	0.520	0.041	0.647
pH	0.022	0.762	0.006	0.941	0.010	0.921
Soil temperature	0.302	**0.026***	0.357	**0.009****	0.307	**0.022***

Note: SOC, soil organic-carbon content; DOC, dissolved organic-carbon content; TN, total nitrogen content; TP, total phosphorus content; AP, available P content; C:N ratio, the ratio of SOC to TN; C:P ratio, the ratio of SOC to TP; N:P ratio, the ratio of TN to TP; Moisture, soil moisture content.
** $P < 0.01$.
* $P < 0.05$.

Table 4
Relationships among dissimilarities of the bacterial, archaeal and fungal communities, environmental factors and geographic distance identified by partial Mantel tests.

Variables	Control for	Mantel statistic r	P
Bacteria			
Environmental factors	Geographic distance	0.247	0.005**
Geographic distance	Environmental factors	0.134	0.047*
Archaea			
Environmental factors	Geographic distance	0.264	0.002**
Geographic distance	Environmental factors	0.155	0.033*
Fungi			
Environmental factors	Geographic distance	0.164	0.027*
Geographic distance	Environmental factors	0.333	0.001***

*** $P < 0.001$.
** $P < 0.01$.
* $P < 0.05$.

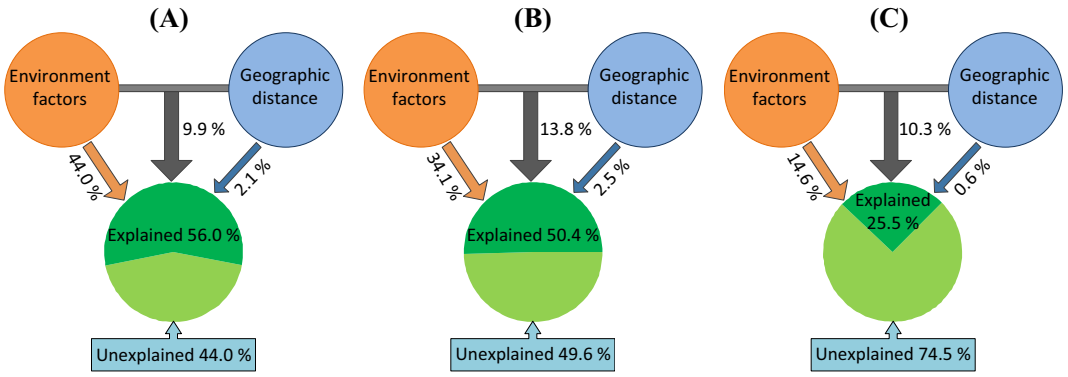

Fig. 6. Variation-partitioning analysis showing the percentages of the variance in the bacterial (A), archaeal (B) and fungal (C) communities explained by the environmental variables and geographic distance. Environmental variables include SOC, soil organic-carbon content; DOC, dissolved organic-carbon content; NO_3^--N content; NH_4^+-N content; TP, total phosphorus content; C:N ratio, the ratio of SOC to TN; C:P ratio, the ratio of SOC to TP; N:P ratio, the ratio of TN to TP; soil temperature.

however, reported that the diversities of the microbial communities were generally lower in the rhizosphere than the bulk soil (Marilley and Aragno, 1999; Ai et al., 2012; Guo et al., 2016). Three possible explanations could account for these inconsistent findings.

Firstly, soil temperature is one of the most important drivers affecting microbial communities (Zhou et al., 2016) and was lower in our study area (mean annual temperature of 4.2 °C) than those in the grassland and agricultural ecosystems (mean annual temperatures of 12.6–14.5 °C) (Table S3). Our low soil temperature could create a relatively stable habitat and lead to low nutrient fluctuation and microbial metabolic activities in the alpine ecosystem, thus contributing to the consistent diversity of the microbial communities between the rhizosphere and bulk soil. Secondly, the soil of the *A. fabri* community had high nutrient and moisture contents in both the rhizosphere and bulk soil (Table S2), which could meet the needs of the microorganisms. Thirdly, the alpine ecosystem would have weaker root activities due to the low temperatures, such as lower absorption of nutrients and secretion of organic acids, so the influence of the root systems on the rhizosphere microbial communities would be weak (Meng et al., 2017). Previous studies found that variations in the composition of microbial communities between the rhizosphere and bulk soil could be controlled by differences in nutrient availability and root selection pressure (Kielak et al., 2010; Ai et al., 2012). We therefore concluded that the soil environment (e.g. low temperature and high nutrient content) was responsible for this consistency of the microbial communities between the rhizosphere and bulk soil in this alpine ecosystem.

4.2. Contrasting drivers of bacterial, archaeal and fungal communities in the alpine ecosystem

The critical environmental factors varied greatly across the altitudinal gradient in the *A. fabri* community on Mount Gongga. Altitude, soil temperature and the C:N ratio had the most influence on the diversity and composition of the microbial communities (Table 3). Altitude was the most important environmental factor, which can affect microbial communities not only by regulating the microclimate and the availability of nutrients but also by conditioning geographic distances (J.T. Wang et al., 2015). Different climatic zones can occur over very short geographic distances due to steep environmental gradients with varied soil properties (Lin et al., 2015). The altitudinal climatic zone in our study area was likely responsible for the variation of the soil microbial communities. Geographic distance was a notable factor shaping the structures of the microbial communities (Table 4 and Fig. 6). Soil bacteria have a limited capacity for long-distance dispersal over broad geographic scales (X.B. Wang et al., 2015), partly due to burial and the cold environment. A previous study found that the dominant archaeal taxa MBGA on the Tibetan Plateau, usually abundant in marine sediments (Inagaki et al., 2006), was due to the historical contingency (geographic distance) of the uplifting of the Tibetan Plateau. The geographic distance along the altitudinal gradient could therefore also greatly affect the patterns of microbial distribution.

Soil temperature was another important factor regulating microbial-community structure. The microbial community structures in the Mount Gongga and the permafrost layer of the Tibetan Plateau (Chen et al., 2017b; Li et al., 2018) and across a range of ecosystems on an intercontinental scale (Zhou et al., 2016) are strongly correlated with temperature. It suggests that soil temperature plays an important role in shaping microbial community structures. The physiological stresses or low temperatures at high altitudes could hinder microbial growth and reduce their diversity (McCain, 2010). Zhou et al. (2016) reported that temperature caused variations in microbial diversities over broad geographic scales, mainly by altering the rates of metabolism, growth and ecosystem productivity. Alternate freezing and thawing can also alter soil microbial communities directly by affecting the metabolic activity and reproduction of soil microbes and indirectly by affecting soil physical properties, such as moisture content and rock weathering (J.T. Wang et al., 2015). The differences and changes of air/soil temperature caused by seasonal freezing and thawing and by altitudinal variations are therefore key ecological factors affecting the structure of soil microbial communities in alpine ecosystems.

The C:N ratio was also significantly correlated with microbial-community structure, as also reported by several studies (Shen et al., 2013; Lin et al., 2015; X.B. Wang et al., 2015). Plants interact with soil microbial communities by the input of litter and root exudates (Knelman et al., 2012; Cui et al., 2018). Plants can determine the sources of soil C and N and alter the soil physical and chemical environments, and thus indirectly affect soil microbial communities (Landesman et al., 2014; Li et al., 2018). The effects of the C:N ratio on the microbial communities in our study, however, were likely not caused by plants due to the consistency of the microbial communities between the rhizosphere and bulk soil, suggesting the importance of soil nutrient stoichiometry in determining microbial-community structure. The impacts of the C:N ratio on the microbial communities may be attributed to the disruption of the elemental stoichiometric balance and homeostasis by the microorganisms (Sinsabaugh et al., 2009). The C:N ratio was thus a good predictor of microbial-community variation. Soil pH has generally been considered a major factor determining microbial diversity and composition (J.T. Wang et al., 2015; Hu et al., 2016; Li et al., 2018), but pH did not have a significant effect in our study (Table 3), perhaps partly because the range of soil pH at our sampling sites was small (3.66–4.39). The lack of neutral to alkaline sites in our study area may partially account for the lack of an effect of soil pH on the microbial communities.

Special factors driving the variation were identified for the

bacterial, archaeal and fungal communities (e.g., DOC and NH_4^+-N contents and C:P and N:P ratios for archaea and NO_3^--N content for fungi) (Table 3 and Fig. 5), supporting our third hypothesis. NH_4^+-N and NO_3^--N are exclusive N resources for bacteria and fungi in agricultural ecosystems and participate in protein synthesis in bacteria and fungi, respectively (Bottomley et al., 2012). Fungi can also acquire C from the decomposition of plant material rich in lignin and cellulose (i.e. lignocellulose) (Meier et al., 2010) and thus need to acquire more soil available N (NO_3^--N) to achieve a biomass elemental stoichiometric balance. Our results further indicated that the archaea and fungi in this alpine ecosystem preferred NH_4^+-N and NO_3^--N, respectively. The archaeal community was also affected by other nutrient properties such as SOC, DOC and nutrient ratios. Archaea have a lower capacity than bacteria and fungi to decompose litter (Singh et al., 2012), and are thus more sensitive to variations in soil nutrients. The different roles of the environmental factors in the microbial communities can further account for the distinct variations of bacteria and archaea along the altitudinal gradient. These results imply the distinct metabolic separation and resource preferences of the bacteria, archaea and fungi in the alpine ecosystem, even over short distances, due to their differential responses to the environmental factors.

4.3. Ecological implications of microbial-community variation in the alpine ecosystem

Our study found highly consistent microbial communities between the rhizosphere and bulk soil represented by the alpha and beta diversities in this alpine ecosystem, in contrast to generally distinct microbial communities between rhizospheres and bulk soil in agricultural and grassland ecosystems (Ai et al., 2012; Guo et al., 2016). Rhizospheres could thus play a relatively weak role in belowground ecological processes and the turnover of soil nutrients at high altitudes where temperatures are low. For example, the metabolic activities of roots are weaker, sediments are fewer and less low-molecular-weight organic matter is secreted by roots in alpine ecosystems than agricultural and grassland ecosystems (Meng et al., 2017). These findings suggest that the belowground ecological processes mediated by roots may not always benefit nutrient cycling in alpine ecosystems, impeding our ability to predict nutrient turnover in these ecosystems sensitive to climate change more than previously thought. The different patterns of microbial diversity along the altitudinal gradient also suggested that the distinctness of the environmental conditions within short distances can profoundly influence microbial metabolic and functional differentiation, implying that ecosystem processes driven by microbes would differ substantially in different microenvironments.

The specific responses of the bacterial, archaeal and fungal communities to soil nutrients further indicated the metabolic diversity of the microbial taxa. For example, *Acidobacteria*, the most abundant bacterial group, were more abundant at 3200–3500 m than at 2800–3200 m (Fig. 3). *Acidobacteria* are generally oligotrophic and versatile heterotrophs (Nemergut et al., 2010), featuring low metabolic rates under low-nutrient conditions (Ward et al., 2009). Their higher abundance in the C-poor soil at high altitudes (SOC and DOC contents are lower at higher altitudes) was consistent with this pattern. *Proteobacteria* were also abundant, and many of the *Proteobacteria* sequences represented *Rhodoplanes* and *Bradyrhizobium*, indicating a potential role of N_2 fixation in the active layer of the Tibetan Plateau (Yarwood et al., 2010; Lin et al., 2015). The breakdown of plant materials rich in lignin and cellulose by fungi is a crucial process in terrestrial C cycling (Meier et al., 2010). These results indicated that the microbial taxa could perform various metabolic functions and have distinct nutrient preferences in the alpine ecosystem and were thus able to tolerate environmental stress and maintain belowground ecological functions under environmental change. We also demonstrated that this high-altitude ecosystem harbored a rich array of soil microbial phyla with varied metabolic characteristics, highlighting the necessity of studying overall microbial taxa, including bacteria, archaea and fungi, to gain a more complete understanding of microbial ecology in alpine ecosystems.

5. Conclusions

This study provides insights into the distributional patterns and drivers of microbial communities in rhizosphere and bulk soil along an altitudinal gradient containing the same vegetation type and improves our understanding of microbial ecology in alpine ecosystems. We found significant differences in microbial alpha diversity and microbial-community structure along the gradient but not between the rhizosphere and bulk soil. Environmental factors (explaining 14.6–44% of the variance) and geographic distance (explaining 0.6–2.5% of the variance) together accounted for most of the microbial-community variations. These findings suggested that edaphic conditions were the main driving factors of microbial-community variation, but geographic distance also played a non-negligible role in microbial-community composition, even along a small-scale altitudinal gradient containing the same vegetation. We also identified specific drivers among the bacterial, archaeal and fungal taxa, suggesting differences and complex responses of the microorganisms to environmental changes in this alpine ecosystem. Our findings also suggest that microorganisms in alpine ecosystems could be less affected by environmental variation and vegetation than microorganisms in other ecosystems due to diverse microbial metabolic strategies and the weak impact of roots.

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Appendix A. Supplementary data

Supplementary information provides additional tables showing the effects of altitude, location (rhizosphere and bulk soil) and their interaction on soil parameters, microbial alpha diversity and the correlations between microbial alpha diversity and the environmental variables. Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2018.11.047.

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